(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number
WO 02/10195 A2

(51) International Patent Classification[7]: C07K 14/00

(21) International Application Number: PCT/CA01/01119

(22) International Filing Date: 2 August 2001 (02.08.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/222,619 2 August 2000 (02.08.2000) US

(71) Applicant *(for all designated States except US)*: **THERATECHNOLOGIES INC.** [CA/CA]; 2310, Alfred-Nobel Boulevard, Saint-Laurent, Québec H4S 2A4 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **GRAVEL, Denis** [CA/CA]; 207, des Pyrénées, St-Lambert, Québec J4S 1L3 (CA). **HABI, Abdelkrim** [CA/CA]; 7961, Champ d'Eau, Anjou, Québec H1J 1X4 (CA). **ABRIBAT, Thierry** [CA/CA]; 4659, Hutchison, Montréal, Québec H2V 4A2 (CA).

(74) Agent: **SWABEY OGILVY RENAULT**; Suite 1600, 1981 McGill College Avenue, Montréal, Québec H3A 2Y3 (CA).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/10195 A2

(54) Title: MODIFIED BIOLOGICAL PEPTIDES WITH INCREASED POTENCY

(57) Abstract: The present invention is concerned with modified biological peptides providing increased potency, prolonged activity and/or increased half-life thereof. The modification is made via coupling through an amide bond with at least one conformationally rigid substituent, either at the N-terminal of the peptide, the C-terminal of the peptide, on a free amino or carboxyl group along the peptide chain, or at a plurality of these sites. Those peptides exhibit clinical usefulness for example in treating states of insulin resistance associated with pathologies such as type II diabetes.

MODIFIED BIOLOGICAL PEPTIDES WITH INCREASED POTENCY

FIELD OF THE INVENTION

The present invention is concerned with modified peptides providing increased biological potency, prolonged activity and/or increased half-life thereof. The modification is made via coupling through an amide bond with at least one conformationally rigid substituent either at the N-terminal of the peptide, the C-terminal of the peptide, or a free amino or carboxyl group along the peptide chain, or at a plurality of these sites.

BACKGROUND OF THE INVENTION

Most peptides are rapidly degraded in a serum medium and as a result, their metabolites may sometimes end up with little or no residual biological activity. To increase the activity of a peptide, various techniques have been proposed. One of them is to anchor a hydrophobic chain at the N- or C-terminal of the peptidic sequence or at other residues along the peptidic chain. This technique nevertheless has limitations. For example, if the peptide comprises a long peptidic chain, the fact that a small hydrophobic group is anchored to the N- or C-terminal does not necessarily result in an increased activity of the peptide so-modified.

For example, it is known that substituting OH for a more hydrophobic group like $-NEt_2$ at the C-terminal of a peptide sequence can result in a significantly increased specific activity. However, these results are contradicted by several

publications, such as Muranichi et al. in *Pharm. Res.*, 1991, 8, 649-652, which stresses the inefficacy of a lauroyl group as a hydrophobic group at the N-terminal to increase activity. Accordingly, there does not seem to be any general rule or conclusion concerning biological potency, duration of activity and/or half life, that can be derived as a result of the addition of substituents on a peptide chain, whether at the N- or C-terminal, or on certain residues along the peptidic chain.

US 6,020,311 discloses a hydrophobic growth hormone-releasing factor (GRF) analog wherein a rigidified hydrophobic moiety is coupled to the GRF peptide via an amide bond at the N-terminal of the peptide. Such analog is said to have an improved anabolic potency with reduced dosage, and a prolonged activity. According to the teaching of this patent, however, the rigidified hydrophobic moiety always comprises a carbonyl group at one extremity, which means that an amide coupling thereof to the GRF can only take place at an amino site to form the required amide bond. The patent does not mention, suggest or imply that similar results could be obtained if the amide coupling was made at the C-terminal by replacing the carbonyl group on the rigidified hydrophobic moiety with an amino group. The patent does not further mention, suggest or imply that the amide coupling could take place elsewhere on the peptide chain.

Biochemistry 2001, 40, pages 2860 to 2869 describes an hydrophobic glucagon-like peptide-1 (GLP-1) analog wherein hexenoic acid, a rigidified hydrophobic moiety is coupled to the GLP-1 peptide at the N-terminal of the peptide. The results show that

this analog exhibits a decreased affinity for the GLP-1 receptor, but an in vivo bioactivity similar to or slightly better than that of the wild type GLP-1, hypothetically because of increased resistance to serum degradation. According to this study, the linkage of acyl chains to His^1, amino-acid substitutions of Ala^2, and the addition of amino-acid sequences at the N-terminal of the molecule would be better strategies to increase the in vivo biological activity than anchoring rigidified hydrophobic chains. However, most of these strategies involve a modification of the amino-acid composition of the natural molecule, which might have negative safety consequences for clinical applications, including the risks for immunogenicity and side effects.

There is therefore a great need to develop peptides modified in a manner such that their activity will be increased, thereby improving their potency, i.e, greater resistance to serum degradation and/or from hyperagonistic properties, and/or increasing their half-life without changing the amino-acid sequence that would be clinically safe and acceptable.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is now provided a peptide of formula X_n-R_1 wherein:

- R_1 is a peptide sequence which cannot be the GRF sequence when X represents a trans-3-hexenoyl group attached at N-terminal position of the peptide sequence;

- each X can be identical or independent from the others and is selected from the following list constituted by conformationally rigid moieties bearing:
  a) a carboxy or an amino group for coupling with the peptide sequence via an amide bond at the N-terminal of the peptide sequence, the C-terminal of the peptide sequence, at an available carboxy or amino site on the peptide sequence chain, and combinations thereof; and
  b) a carboxy group for coupling with the peptide sequence via an ester bond at an available hydroxy site on the peptide sequence chain, and combinations thereof;

wherein,

n is any digit between 1 to 5;

X being defined as:

i) a straight, substituted C_1-C_{10} alkyl;

ii) a branched, substituted C_1-C_{10} alkyl;

iii) a straight or branched, unsubstituted or substituted C_1-C_{10} alkene;

iv) a straight or branched, unsubstituted or substituted C_1-C_{10} alkyne;

v) an unsubstituted or substituted, saturated or unsaturated C_3-C_{10} cycloalkyl or heterocycloalkyl wherein the heteroatom is O, S or N;

vi) an unsubstituted or substituted C_5-C_{14} aryl or heteroaryl wherein the heteroatom is O, S or N;

wherein the substituent in the definitions i) to vi) comprises one or more

a) straight or branched C_1-C_6 alkyl;

b) straight or branched C_1-C_6 alkene;

c) straight or branched C_1-C_6 alkyne;

d) C_3-C_{10} cycloalkyl or heterocycloalkyl wherein at least 2 carbon atoms are optionally connected to the C_1-C_{10} alkyl, C_1-C_{10} alkene, C_1-C_{10} alkyne, C_3-C_{10} cycloalkyl or heterocycloalkyl, and C_5-C_{14} aryl or heteroaryl; or

e) C_5-C_{14} aryl or heteroaryl wherein at least 2 carbon atoms of the aryl or heteroaryl are optionally connected to the C_1-C_{10} alkyl, C_1-C_{10} alkene, C_1-C_{10} alkyne, C_3-C_{10} cycloalkyl or heterocycloalkyl, and C_5-C_{14} aryl or heteroaryl;

and any isomers thereof, including cis and trans configurations, epimers, enantiomers, diastereoisomers, and racemic mixtures.

The term "aryl" includes phenyl, naphtyl and the like; the term "heterocycloalkyl" includes tetrahydrofuranyl, tetrahydrothiophanyl, tetrahydrothiopyranyl, tetrahydropyranyl and partially dehydrogenated derivatives thereof, azetidinyl, piperidinyl, pyrrolidinyl, and the like; the term "heteroaryl" comprises pyridinyl, indolyl, furanyl, imidazolyl, thiophanyl, pyrrolyl, quinolinyl, isoquinolinyl, pyrimidinyl, oxazolyl, thiazolyl, isothiazolyl, isooxazolyl, pyrazolyl, and the like.

The expression "conformationally rigid moiety" means an entity having limited conformational, i.e., rotational, mobility about its single bonds. Such mobility is limited, for example, by the presence of a double bond, a triple bond, or a saturated or unsaturated ring, which have little or no conformational mobility. As a result, the number of conformers or rotational isomers is reduced when compared, for

example, with the corresponding straight, unsubstituted and saturated aliphatic chain. The conformationally rigid moiety may be hydrophobic, although this is not a prerequisite.

According to a preferred embodiment of the present invention the peptide sequence is selected from the group consisting of Growth hormone releasing factor (GRF), Somatostatin, Glucagon-like peptide 1 (7-37), amide human (GLP-1), hGLP-1 (7-36) NH_2 Parathyroid hormone fragments such as (PTH 1-34), Adrenocorticotropic hormone (ACTH), Osteocalcin, Calcitonin, Corticotropin releasing factor, Dynorphin A, β–Endorphin, Big Gastrin-1, GLP-2, Luteinizing hormone-releasing hormone, Melanocyte Stimulating Hormone (MSH), Atrial Natriuretic Peptide, Neuromedin B, Human Neuropeptide Y, Human Orexin A, Human Peptide YY, Human Secretin, Vasoactive Intestinal peptide (VIP), Antibiotic peptides (Magainin 1, Magainin 2, Cecropin A, and Cecropin B), Substance P (SP), Beta Casomorphin-5, Endomorphin-2, Procolipase, Enterostatin, gastric inhibitory peptide, Chromogranin A, Vasostatin I & II, Procalcitonin, ProNCT, ProCGRP, IL8 (monocyte-derived), GCP-2, PF4, IP-10, MIG, SDF-1α, GRO-α, I-TAC, RANTES, LD78, MIP-1α, MCP-1, MCP-2, MCP-3, MCP-4, Eotaxin, MDC, and functional derivatives or fragments thereof.

DETAILED DESCRIPTION OF THE INVENTION

The amino acids are identified in the present application by the conventional three-letter abbreviations as indicated below, which are as generally

accepted in the peptide art as recommended by the IUPAC-IUB commission in biochemical nomenclature:

Alanine	Ala	Leucine	Leu
Arginine	Arg	Lysine	Lys
Asparagine	Asn	Methionine	Met
Aspartic acid	Asp	Phenylalanine	Phe
Cysteine	Cys	Proline	Pro
Glutamic acid	Glu	Serine	Ser
Glutamine	Gln	Threonine	Thr
Glycine	Gly	Tryptophan	Trp
Histidine	His	Tyrosine	Tyr
Isoleucine	Ile	Valine	Val

All the peptide sequences set out herein are written according to the generally accepted convention whereby the N-terminal amino acid is on the left and the C-terminal amino acid is on the right.

The present invention relates to the use of at least one conformationally rigid moiety, to produce a new family of peptides with enhanced pharmacological properties.

The modified peptides of the present invention are prepared according to the following general method, well known in the art of solid phase synthesis.

Conformationally rigid moieties comprising a carboxy group are used for anchoring to amino groups such as those found on the lysine side chain as well

as the N-terminus of peptides. Those comprising an amino group are used for anchoring to carboxyl groups such as those found on the aspartic or glutamic acid side chains or the C-terminus of peptides. For such cases, the anchoring reaction is preferably performed on a solid phase support (Merrifield R.B. 1963, *J. Am. Chem. Soc.*,1963, 85, 2149 and *J. Am. Chem. Soc.*, 1964, 86, 304) using Benzotriazole-1-yl-oxy-tris (dimethylamino) phosphonium hexafluorophosphate described by Castro in the article (B. Castro et al., 1975, *Tetrahedron letters*, Vol. 14 :1219).

With respect to the anchoring dynamic, the preferred working temperatures are between 20°C and 60°C. The anchoring reaction time in the case of the more hydrophobic moieties, varies inversely with temperature, and varies between 0.1 and 24 hours.

Synthesis steps were carried out by solid-phase methodology on a manual peptide synthesizer using the Fmoc strategy. Fmoc amino acids were supplied by Chem Impex International Inc. Chicago and other commercial sources. Sequential Fmoc chemistry using BOP as coupling reagent was applied to the PL-Wang resin (Polymer Laboratories, catalog number : 1463-4799) for the production of the C-terminal carboxylic acid.

Fmoc deprotections were accomplished with piperidine 20% solution in DMF in three consecutive steps. Always under nitrogen scrubbing, a first solution of piperidine 20% was used for 1min. to remove the major part of the Fmoc

protecting groups. Then, the solution was drained, and another fresh piperidine 20% solution was introduced this time for 3min., drained again and finally another solution of piperidine 20% for 10min. The peptide-resin was then washed 4 times successively with 50 mL of DMF under nitrogen scrubbing. After completion of the synthesis, the resin was well washed with DMF and DCM prior to drying.

Final cleavage of side chain protecting groups and peptide-resin bonds were performed using the following mixture: TFA, ethanedithiol, triisopropylsilane, thioanisole, phenol, water (92 :1.66 :1.66 :1.66 :1 :2). A final concentration of 20 mL of cleavage cocktail per gram of dried peptide-resin was used to cleave the peptide from the resin. The cleavage reaction was performed at room temperature for 2 hours. The free peptide, now in solution in the TFA cocktail, was then filtered on a coarse fritted disk funnel. The resin was then washed 3 times with pure TFA. The peptide/TFA mixture was evaporated under vacuum on a Rotary evaporator, precipitated and washed with ether prior to its dissolution in water and freeze drying to eliminate the remaining traces of solvent and scavengers.

Coupling of the first Fmoc-amino acid to the Wang resin

We used 4-alkoxybenzyl alcohol polystyrene (Wang resin) and 2 eq of the desired Fmoc-amino acid in DMF and let both products mix together under nitrogen scrubbing for 15min at room temperature. Then 3.3 eq of pyridine and 2 eq of 2,6-dichlorobenzoylchloride were added successively and the reaction was

carried out under nitrogen scrubbing for 15-20 hours. (Seiber P., 1987, *Tetrahedron Letters*, Vol. 28, No. 49, pp 6147-6150). After this reaction, the reaction vessel was drained and the resin washed 4 times successively with DMF under nitrogen scrubbing. Any remaining hydroxyl groups of the resin were benzoylated with 3 eq of benzoylchloride and pyridine in DCE (dichloroethane) for 2 hours.

Coupling of each remaining amino acid on the growing peptide

For each of the following Fmoc-amino acid we dissolved 3 eq of the Fmoc-amino acid with 3 eq of BOP (Benzotriazole-1-yl-oxy-tris (dimethylamino) phosphonium hexafluorophosphate) (B. Castro et al., 1975, *Tetrahedron letters*, Vol. 14 :1219) in DMF, added the resulting solution to the resin in the reaction vessel, started the nitrogen scrubbing and added 6 eq of DIPEA (diisopropylethylamine) to start the coupling reaction. The coupling mixture was scrubbed under nitrogen for 60 min. in the reaction vessel; then drained from the vessel, the resin was washed 3 times successively with DMF and a qualitative ninhydrin test was performed to verify completion of the reaction.

The coupling of the Fmoc-L-Lys(Aloc)-OH (PerSeptive Biosystems, catalog number : GEN911209), Fmoc-L-Glu(OAl)-OH (PerSeptive Biosystems, catalog number : GEN911207) and Fmoc-L-Asp(OAl)-OH (PerSeptive Biosystems, catalog number : GEN911205) were carried out in the same way as for the Fmoc-amino acids as described above.

Deprotection of allylic groups

The peptide-resin (X mmol) was then introduced in DCM under nitrogen scrubbing and after 10 min. the $PdCl_2(PPh_3)_2$ (X mmol x 0.05 / 0.05 eq) (palladium(II) bis-triphenylphosphine) was added to the mixture (Bürger H., Kilion W., *J. Organometallics*, 1969, 18:299). Then the $(CH_3CH_2CH_2)_3SnH$ (X mmol x 6 / 6eq) (tributyltinhydride) was diluted in DCM and added dropwise to the peptide-resin suspension with an addition funnel over a period of 30 minutes. The reaction was continued for another 10 minutes then the vessel was drained from the cleavage mixture and right after the peptide-resin was washed 4 times with DCM and 4 times with DMF (Dangles O., Guibé F., Balavoine G., Lavielle S., Marquet A., 1987, *J. Org. Chem.*, 52: 4984).

Coupling of the conformationally rigid acids and alkylamines

The coupling of the conformationally rigid acids and amines to the side chains of the peptide-resin was conducted under the same conditions as those of the Fmoc-amino acids except that for these side chain modifications we used 10 equivalents of the rigid moieties and coupling reagent instead of 3.

The invention is not limited to any particular peptide sequence. Preferred peptide sequences R^1 comprise those with therapeutic properties, as well as functional derivatives or fragments thereof. The therapeutic properties of such peptides which may be used in accordance with the present invention include,

without limitation, treatment of bone diseases including osteoporosis, postmenopausal osteoporosis and bone deposits, cancer treatment, regulating blood glucose, type II diabetes, treatment to to enhance mucosal regeneration in patients with intestinal diseases, treatment for diseases related to inflammatory responses, obesity treatment, treatment for autism and pervasive development disorders, hyperproliferative skin conditions, aging, altering the proliferation of peripheral blood mononuclear cells, regulation of myometrial contractility and of prostaglandin release, stimulation of ACTH release, inhibition of interleukin-8 production, stimulation of acid release, enhancement of mucosal regeneration in patients with intestinal diseases, treatment for hormone-dependent diseases and conditions including for hormone-dependent cancers, modulation of melanocyte information process, involved in pressure and volume homeostasis, regulation of exocrine and endocrine secretions, smooth muscle contraction, feeding, blood pressure, blood glucose, body temperature and cell growth, regulation of food intake and energy balance, inhibition of cancer cell growth, stimulation of pancreatic secretion, or stimulate cell growth.

Growth hormone releasing factor (GRF):

Xaa_1-Xaa_2-Asp-Ala-Ile-Phe-Thr-Xaa_8-Ser-Tyr-Arg-Lys-Xaa_{13}-Leu-Xaa_{15}-Gln-Leu-Xaa_{18}-Ala-Arg-Lys-Leu-Leu-Xaa_{24}-Xaa_{25}-Ile-Xaa_{27}-Xaa_{28}-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu-NH_2

wherein,

Xaa$_1$ is Tyr or His;
Xaa$_2$ is Val or Ala;

Xaa_8 is Asn or Ser;
Xaa_{13} is Val or Ile;
Xaa_{15} is Ala or Gly;
Xaa_{18} is Ser or Tyr;
Xaa_{24} is Gln or His;
Xaa_{25} is Asp or Glu;
Xaa_{27} is Met, Ile or Nle; and
Xaa_{28} is Ser or Asn.

Somatostatin:

Ala_1-Gly-Cys-Lys-Asn-Phe-Phe-Trp
|
Cys_{14}-Ser-Xaa_{12}-Phe-Thr-Lys
|

wherein,

Xaa_{12} is Tyr or Ser.

Glucagon-like peptide 1 (7-37), (amide human (hGLP-1)):

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly-OH(NH_2)

Parathyroid hormone fragments (PTH 1-34):

Xaa_1-Val-Ser-Glu-Xaa_5-Gln-Xaa_7-Met-His-Asn-Leu-Gly-Xaa_{13}-His-Xaa_{15}-Xaa_{16}-Xaa_{17}-Xaa_{18}-Glu-Arg-Xaa_{21}-Xaa_{22}-Trp-Leu-Xaa_{25}-Xaa_{26}-Lys-Leu-Gln-Asp-Val-His-Xaa_{33}-Xaa_{34}-NH_2

wherein,

Xaa_1 is Ser or Ala;

Xaa_5 is Ile or Met;

Xaa_7 is Leu or Phe;

Xaa_{13} is Lys or Glu;

Xaa_{15} is Leu or Arg;

Xaa_{16} is Asn or Ala or Ser or His;

Xaa_{17} is Ser of Thr;

Xaa_{18} is Met or Val or Leu;

Xaa_{21} is Val or met or Gln;

Xaa_{22} is Glu or Gln or Asp;

Xaa_{25} is Arg or Gln;

Xaa_{26} is Lys or Met;

Xaa_{33} is Asn or Ser; and

Xaa_{34} is Phe or Ala.

Adrenocorticotropic hormone (ACTH):

Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Xaa_{13}-Gly-Xaa_{15}-Lys-Arg-Arg-Pro-Xaa_{20}-Lys-Val-Tyr-Pro-Asn-Xaa_{26}-Xaa_{27}-Xaa_{28}-Xaa_{29}-Glu-Xaa_{31}-Xaa_{32}-Glu-Xaa_{34}-Xaa_{35}-Xaa_{36}-Xaa_{37}-Glu-Xaa_{39}-NH_2

wherein,

Xaa_{13} is Val or Met;

Xaa_{15} is Lys or Arg;

Xaa_{20} is Val or Ile;

Xaa_{26} is Gly or Ser;

Xaa_{27} is Ala or Phe or Val;

Xaa_{28} is Glu or Gln;

Xaa_{29} is Asp or Asn or Glu;

Xaa_{31} is Ser or Thr;

Xaa_{32} is Ala or Val or Ser;

Xaa_{34} is Ala or Asn or Gly;

Xaa_{35} is Phe or Met;

Xaa_{36} is Pro or Gly;

Xaa_{37} is Leu or Val or Pro; and

Xaa_{39} is Phe or Val or Leu.

Osteocalcin:

Tyr-Leu-Xaa_{52}-Xaa_{53}-Xaa_{54}-Leu-Gly-Ala-Pro-Xaa_{59}-Pro-Tyr-Pro-Asp-Pro-Leu-Glu-Pro-Xaa_{68}-Arg-Glu-Val-Cys-Glu-Leu-Asn-Pro-Xaa_{77}-Cys-Asp-Glu-Leu-Ala-Asp-His-Ile-Gly-Phe-Gln-Xaa_{89}-Ala-Tyr-Xaa_{92}-Arg-Xaa_{94}-Tyr-Gly-Xaa_{97}-Val-NH_2

wherein,

Xaa_{52} is Tyr or Asp or Asn;

Xaa_{53} is Gln or His or Asn;

Xaa_{54} is Trp or Gly;

Xaa_{59} is Val or Ala;

Xaa_{68} is Arg or Lys or His;

Xaa_{77} is Asp or Asn;

Xaa_{89} is Glu or Asp;

Xaa_{92} is Arg or Lys;

Xaa_{94} is Phe or Ile; and

Xaa_{97} is Pro or Thr.

Calcitonin:

Cys-Xaa_{86}-Xaa_{87}-Leu-Ser-Thr-Cys-Xaa_{92}-Leu-Gly-Xaa_{95}-Xaa_{96}-Xaa_{97}-Xaa_{98}-Xaa_{99}-Xaa_{100}-Xaa_{101}-Xaa_{102}-Xaa_{103}-Xaa_{104}-Thr-Xaa_{106}-Xaa_{107}-Xaa_{108}-Xaa_{109}-Xaa_{110}-Xaa_{111}-Gly-Xaa_{113}-Xaa_{114}-Xaa_{115}-Pro-NH_2

wherein,

Xaa_{86} is Gly or Ser or Ala;

Xaa_{87} is Asn or Ser;

Xaa_{92} is Met or Val;

Xaa_{95} is Thr or Lys;

Xaa_{96} is Tyr or Leu;

Xaa_{97} is Thr or Ser;

Xaa_{98} is Gln or Lys;

Xaa_{99} is Asp or Glu;

Xaa_{100} is Phe or Leu;

Xaa_{101} is Asn or His;

Xaa_{102} is Lys or Asn;

Xaa_{103} is Phe or Leu;

Xaa_{104} is His or Gln;

Xaa_{106} is Phe or Tyr;

Xaa_{107} is Pro or Ser;

Xaa_{108} is Gln or Gly or Arg;

Xaa_{109} is Thr or Ile;

Xaa_{110} is Ala or Gly or Ser or Asp or Asn;

Xaa_{111} is Ile or Phe or Val or Thr;

Xaa_{113} is Val or Ala or Ser;

Xaa_{114} is Gly or Glu; and

Xaa_{115} is Ala or Thr.

Corticotropin releasing factor:

Ser-Glu-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Xaa_{101}-Xaa_{102}-Ala-Glu-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Met-Glu-Ile-Ile-NH_2

wherein,

Xaa_{101} is Ala or Pro; and

Xaa_{102} is Arg or Gly.

Dynorphin A:

H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH

β-Endorphin:

H-Tyr-Gly-Gly-Phe-Met-Thr-Xaa_{243}-Glu-Xaa_{245}-Ser-Gln-Thr-Pro-Leu-Xaa_{251}-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Xaa_{259}-Lys-Asn-Xaa_{262}-Xaa_{263}-Lys-Lys-Gly-Xaa_{267}-OH

wherein,

Xaa_{243} is Ser or Pro;

Xaa_{245} is Lys or Arg;

Xaa_{251} is Val or Met;

Xaa_{259} is Ile or Val;

Xaa_{262} is Ala or Thr or Ser or Val;

Xaa_{263} is Tyr or His; and

Xaa_{267} is Glu or Leu or Gln or His.

Big Gastrin-1:

pXaa$_{59}$-Leu-Gly-Xaa$_{62}$-Gln-Xaa$_{64}$-Xaa$_{65}$-Xaa$_{66}$-Xaa$_{67}$-Xaa$_{68}$-Xaa$_{69}$-Ala-Asp-Xaa$_{72}$-Xaa$_{73}$-Lys-Lys-Xaa$_{76}$-Xaa$_{77}$-Pro-Xaa$_{79}$-Xaa$_{80}$-Glu-Xaa$_{82}$-Glu-Glu-Xaa$_{85}$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2

wherein,

Xaa_{59} is Glu or Gln;

Xaa_{62} is Pro or Leu;

Xaa_{64} is Gly or Asp;

Xaa_{65} is Pro or Ser;

Xaa_{66} is Pro or Gln;

Xaa_{67} is His or Gln;

Xaa_{68} is Leu or Met or Phe or Gln;

Xaa_{69} is Val or Ile;

Xaa_{72} is Pro or Leu;

Xaa_{73} is Ser or Ala;

Xaa_{76} is Gln or Glu;

Xaa_{77} is Gly or Arg;

Xaa_{79} is Trp or Pro or Arg;

Xaa_{80} is Leu or Val or Met;

Xaa_{82} is Glu or Lys; and

Xaa_{85} is Glu or Ala.

GLP-2:

His-Ala-Asp-Gly-Ser-Phe-Xaa_{152}-Xaa_{153}-Xaa_{154}-Xaa_{155}-Xaa_{156}-Xaa_{157}-Xaa_{158}-Leu-Asp-Xaa_{161}-Xaa_{162}-Ala-Xaa_{164}-Xaa_{165}-Xaa_{166}-Phe-Xaa_{168}-Xaa_{169}-Trp-Xaa_{171}-Xaa_{172}-Xaa_{173}-Thr-Xaa_{175}-Xaa_{176}-Xaa_{177}-Xaa_{178};

wherein,

Xaa_{152} is Ser or Thr;

Xaa_{153} is Asp or Ser;

Xaa_{154} is Glu or Asp;

Xaa_{155} is Met or Phe;

Xaa_{156} is Asn or Ser;

Xaa_{157} is Thr or Lys;

Xaa_{158} is Ile or Val or Ala;

Xaa_{161} is Asn or Ile or His or Ser;

Xaa_{162} is Leu or Lys;

Xaa_{164} is Ala or Thr;

Xaa_{165} is Arg or Gln or Lys;

Xaa_{166} is Asp or Glu;

Xaa_{168} is Ile or Leu;

Xaa_{169} is Asn or Asp;

Xaa_{171} is Leu or Ile;

Xaa_{172} is Ile or Leu;

Xaa_{173} is Gln or Asn or His;

Xaa_{175} is Lys or Pro;

Xaa_{176} is Ile or Val;

Xaa_{177} is Thr or Lys; and

Xaa_{178} is Asp or Glu.

Luteinizing hormone-releasing hormone:

Xaa_1-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-OH

wherein,

Xaa_1 is pGlu, 5-oxoPro or Gln.

Melanocyte Stimulating Hormone (MSH):

Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2

Atrial Natriuretic Peptide:

H-Ser-Leu-Arg-Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Xaa_{135}-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Xaa_{142}-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr-OH

wherein,

Xaa_{135} is Met or Ile; and

Xaa_{142} is Gly or Ser.

Neuromedin B:

H-Gly-Asn-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH_2

Human Neuropeptide Y:

H-Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-asp-Met-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2

Human Orexin A:

pGlu-Pro-Leu-Pro-Asp-Cys-Cys-Arg-Gln-Lys-Thr-Cys-Ser-Cys-Arg-Leu-Tyr-Glu-Leu-Leu-His-Gly-Ala-Gly-Asn-His-Ala-Ala-Gly-Ile-Leu-Thr-Leu-NH_2

Human Peptide YY:

H-Tyr-Pro-Ile-Lys-Pro-Glu-Ala-Pro-Gly-Glu-Asp-Ala-Ser-Pro-Glu-Glu-Leu-Asn-Arg-Tyr-Tyr-Ala-Ser-Leu-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2

Human Secretin:

H-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Glu-Gly-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH_2

Vasoactive Intestinal peptide (VIP):

H-His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2

Antibiotic peptides such as:

Magainin 1:

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Gly-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-Met-Lys-Ser

Magainin 2:

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-Met-Asn-Ser

Cecropin A:

Lys-Trp-Lys-Val-Phe-Lys-Lys-Ile-Glu-Lys-Val-Gly-Gln-Ala-Thr-Gln-Ile-Ala-Lys

Cecropin B:

Lys-Trp-Lys-Val-Phe-Lys-Lys-Ile-Glu-Lys-Met-Gly-Arg-Asn-Ile-Arg-Asn-Gly-Ile-Val-Lys-Ala-Gly-Pro-Ala-Ile-Ala-Val-Leu-Gly-Glu-Ala-Lys-Ala-Leu.

Substance P (SP):

Arg-Pro-Leu-Pro-Gln-Glu-Phe-Phe-Gly-Leu-Met-amide

Beta Casomorphin-5:

Tyr-Pro-Phe-Pro-Gly

Endomorphin-2:

Tyr-Pro-Phe-Phe-NH2

Procolipase:

100 aa peptide (X1-Pro-X2-Pro-Arg....)

Enterostatin:

Val-Pro-Asp-Pro-Arg

Gastrin Inhibitory Peptide:

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala- Met-Asp-Lys-Ile-His-Gln-Gln-Asp-Phe- Val-Asn-Trp-Leu- Leu-Ala-Gln-Lys-Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn- Ile-Thr-Gln

Chromogranin A

Vasostatin I

Vasostatin II:

Leu Pro Val Asn Ser Pro Met Asn Lys Gly Asp Thr Glu Val Met Lys Cys Ile Val Glu Val Ile Ser Asp Thr Leu Ser Lys Pro Ser Pro Met Pro Val Ser Gln Glu Cys Phe Glu Thr Leu Arg Gly Asp Glu Arg Ile Leu Ser Ile Leu Arg His Gln Asn Leu Leu Lys Glu Leu Gln Asp Leu Ala Leu Gln Gly Ala Lys Glu Arg Ala His Gln Gln Lys Lys His Ser Gly Phe Glu Asp Glu Leu Ser Glu Val Leu Glu Asn

Gln Ser Ser Gln Ala Glu Leu Lys Glu Ala Val Glu Glu Pro Ser Ser Lys Asp Val Met Glu

Procalcitonin

ProNCT

ProCGRP

Chemokine family:

CXC-group:

IL8(monocyte-derived):

SerAlaLysGluLeuArgCysGlnCys...

GCP-2:

GlyProValSerAlaValLeuThrGluLeuArgCysThrCys...

PF4:

GluAlaGluGluAspGlyAspLeuGlnCysLeuCys...

IP-10:

ValProLeuSerArgThrValArgCCysThrCys...

MIG:

ThrProValValArgLysGlyArgCysSerCys...

SDF-1α:

LysProValSerLeuSerTyrArgCysProCys...

GRO-α:

AlaProLeuAlaThrGluLeuArgCysGlnCys...

I-TAC:

PheProMetPheLysLysGlyArgCysLeuCys...

CC-group:

RANTES:

SerProTyrSerSerAspThrThrProCys...

LD78:

AlaProLeuAlaAlaAspThrProThrAlaCys...

MIP-1α:

AlaProMetGlySerAspProProThrAlaCys...

MCP-1:

GlnProAspAlaIleAsnAlaProValThrCys...

MCP-2:

GlnProSerAspValSerIleProIleThrCys...

MCP-3:

GlnProValGlyIleTAsnSeerThrThrCys...

MCP-4:

GlnProAspAlaLeuAspValProSerThrCys...

Eotaxin:

GlyProAlaSerValProThrThrCys...

MDC:

GlyProTyrGlyAlaAsnMetGluAspSerValCys...

and functional derivatives or fragments thereof.

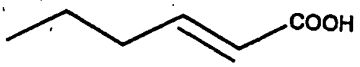
The complete definition of the previously listed sequences are known inter alia from Mentlein, R (1999) Regul. Pept. 85:9-24 and from De Meester, I. Et al. (2000) Adv

ExpMed Biol. 477:67-87. Those documents are incorporated by reference to the present application.

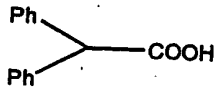
In a more preferred embodiment, the peptide is substituted with one or more conformationally rigid moieties. Preferred structures of the conformationally rigid moieties comprise those with a double bond, a triple bond or a saturated or unsaturated ring.

The following is a brief list of the formula of preferred conformationally rigid moieties, identified as Formula 1 to 63, which are suitable for the purposes of the present invention.

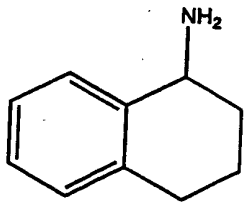
Among the preferred modified peptides according to the present invention, are those wherein the peptide sequence is the sequence of a natural peptide.



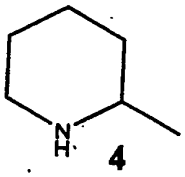
1



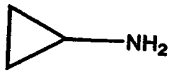
2



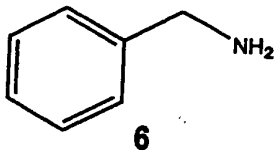
3



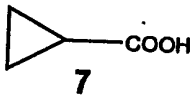
4



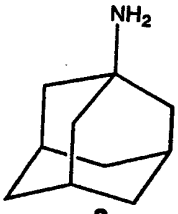
5



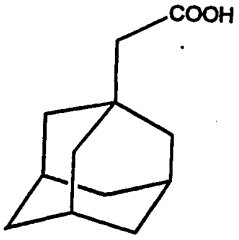
6



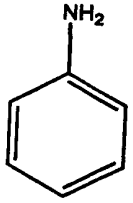
7



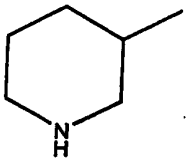
8



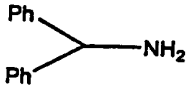
9



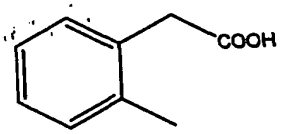
10



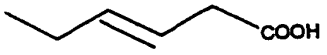
11



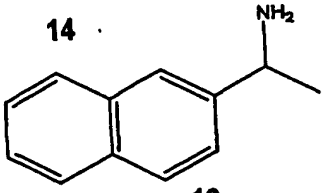
12



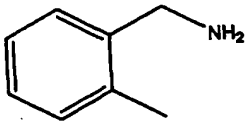
13



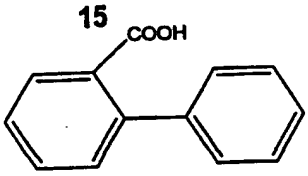
14



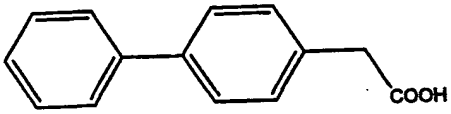
16



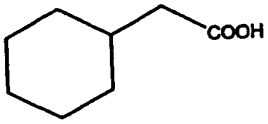
15



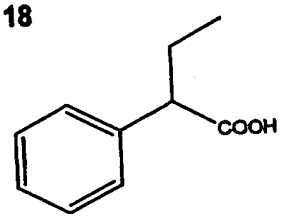
17



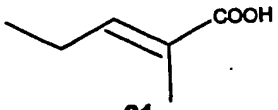
18



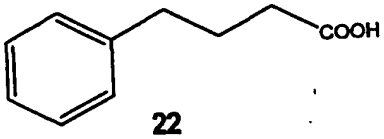
19



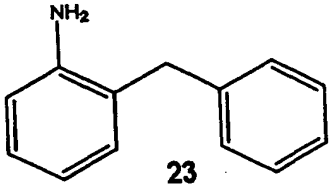
20



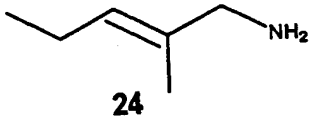
21



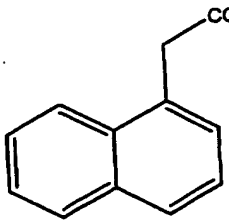
22



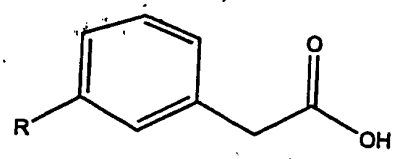
23



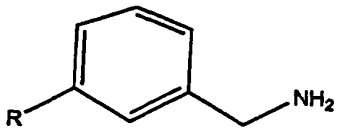
24



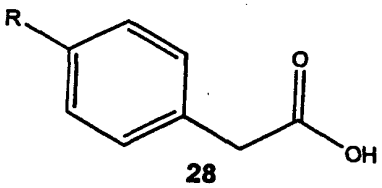
25



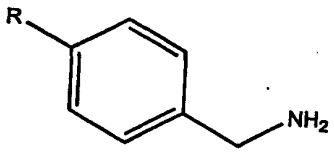
26



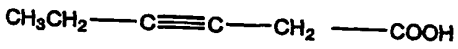
27



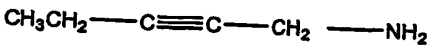
28



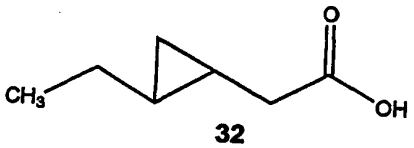
29



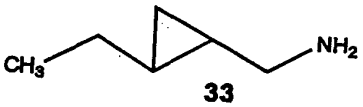
30



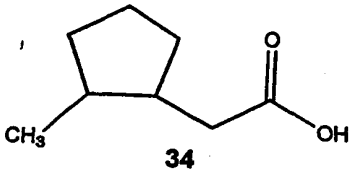
31



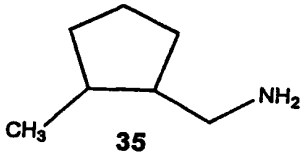
32



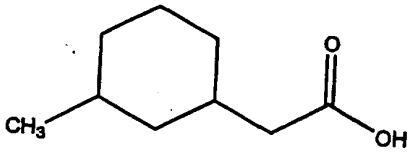
33



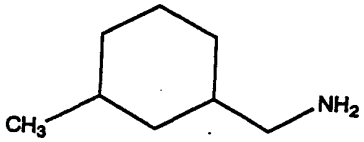
34



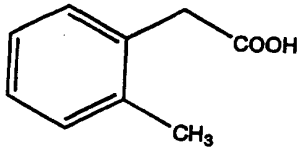
35



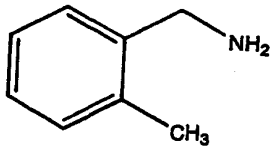
36



37



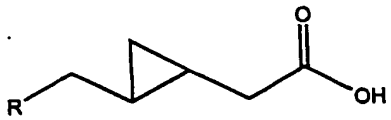
38



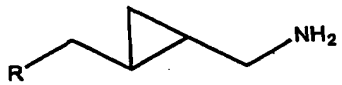
39

40

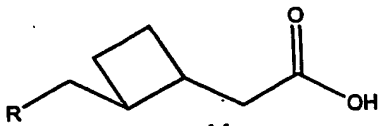
41



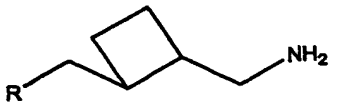
42



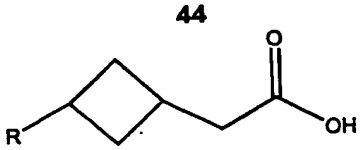
43



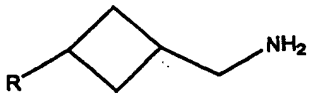
44



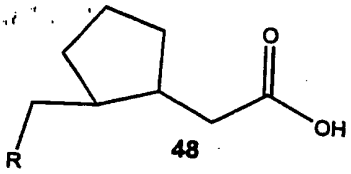
45



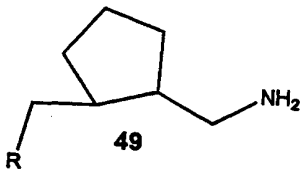
46



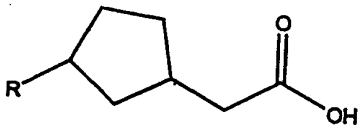
47



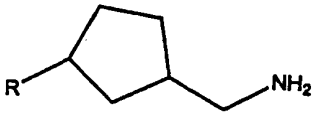
48



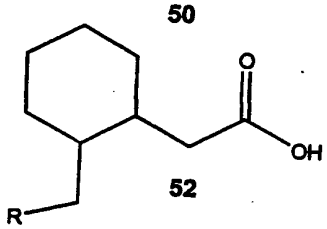
49



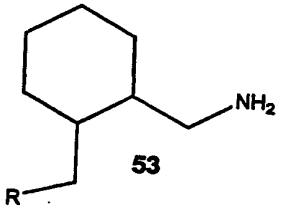
50



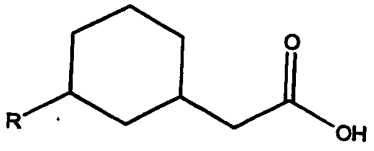
51



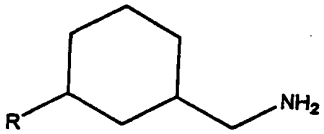
52



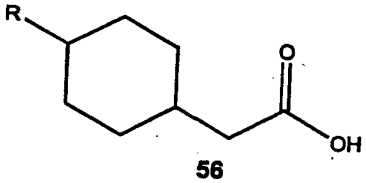
53



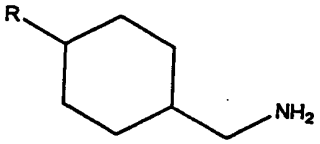
54



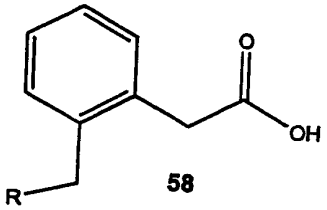
55



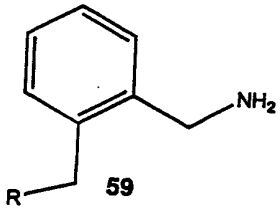
56



57



58

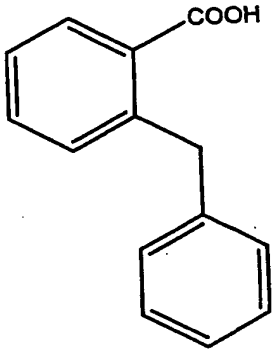


59

60

61

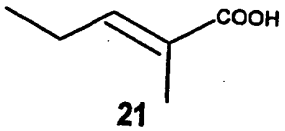
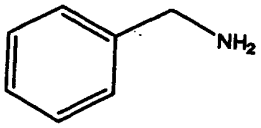
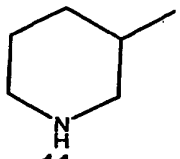
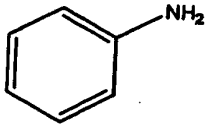
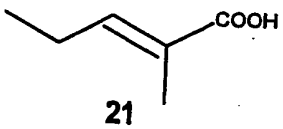
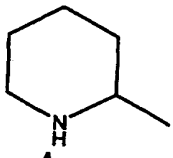
62



63

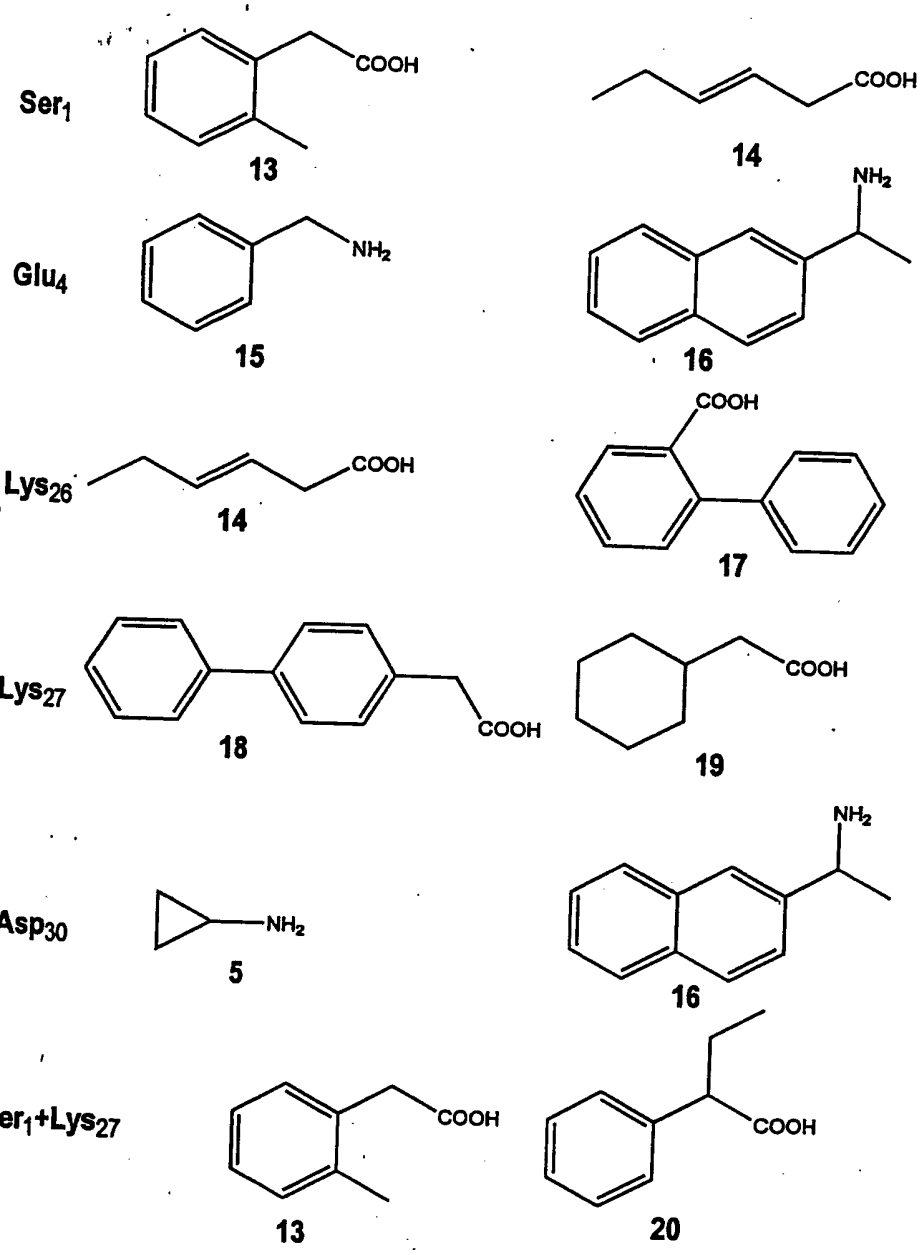
wherein, R is hydrogen, CH_3 or CH_2CH_3.

A preferred embodiment of the present invention is constituted by peptides wherein the peptide sequence is Somatostatin and at least one conformationally rigid moiety is coupled with said somatostatin peptide sequence via an amide bond at different positions as follows:

Position	conformationally rigid moieties
Ala_1	 21
Asp_5	 15  11
Cys_{14}	 10
Ala_1+Cys_{14}	 21  4

An another preferred embodiment of the present invention is constituted by those peptides wherein the peptide sequence is PTH 1-34 and at least one conformationally rigid moiety is coupled with said PTH 1-34 peptide sequence via an amide bond at different positions as follows:

Position	**conformationally rigid moieties**

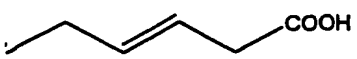


A further preferred embodiment of the present invention is constituted by those peptides wherein the peptide sequence is GLP-1 and at least one

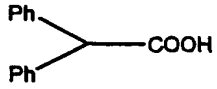
conformationally rigid moiety is coupled with said GLP-1 peptide sequence via an amide bond at different positions as follows:

Position **conformationally rigid moieties**

His_1

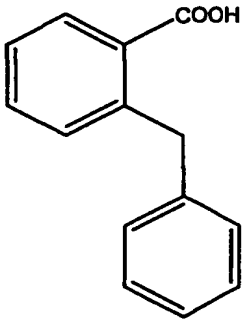


14

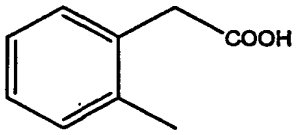


2

60

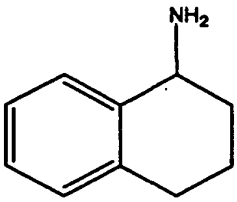


63

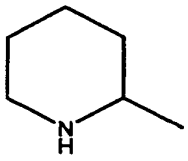


13

Glu_3

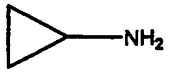


3

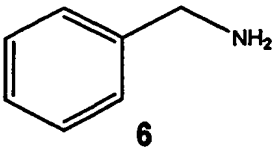


4

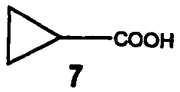
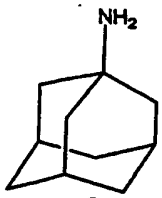
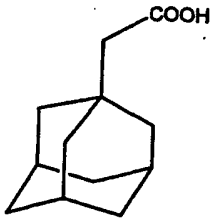
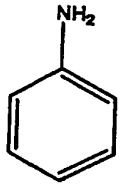
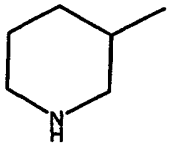
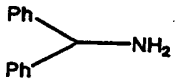
Asp_9



5



6

Position	conformationally rigid moieties	
$His_1 + Glu_3$	 7	 8
$His_1 + Asp_9$	 9	 10
$Glu_3 + Asp_9$	 11	 12

Also preferred among the modified peptides according to the invention are those peptides wherein;

- the peptide sequence is GLP-2 and at least one conformationally rigid moiety is coupled with said GLP-2 peptide sequence via an amide or ester bond at different positions of the peptide sequence;
- the peptide sequence is Enterostatin and at least one conformationally rigid moiety is coupled with said Enterostatin peptide sequence via an amide bond at different positions of the peptide sequence;
- the peptide sequence is NPY and at least one conformationally rigid moiety is coupled with said NPY peptide sequence via an amide or ester bond at different positions of the peptide sequence;
- the peptide sequence is NPYY and at least one conformationally rigid moiety is coupled with said NPYY peptide sequence via an amide or ester bond at different positions of the peptide sequence;
- the peptide sequence is Secretin and at least one conformationally rigid moiety is coupled with said Secretin peptide sequence via an amide or ester bond at different positions of the peptide sequence;
- the peptide sequence is Vasoactive Intestinal Peptide and at least one conformationally rigid moiety is coupled with said Vasoactive Intestinal Peptide sequence via an amide or ester bond at different positions of the peptide sequence;

- the peptide sequence is Gastrin Inhibitory Peptide and at least one conformationally rigid moieties is coupled with said Gastrin Inhibitory Peptide sequence via an amide or ester bond at different positions of the peptide sequence;
- the peptide sequence is Vasostatin II and at least one conformationally rigid moiety is coupled with said Vasostatin II peptide sequence via an amide or ester bond at different positions of the peptide sequence;
- the peptide sequence is RANTES and at least one conformationally rigid moiety is coupled with said RANTES peptide sequence via an amide or ester bond at different positions of the peptide sequence;
- the peptide sequence is Eotaxin and at least one conformationally rigid moiety is coupled with said Eotaxin peptide sequence via an amide or ester bond at different positions of the peptide sequence.

In the modified peptites of the invention, the conformationally rigid moiety is preferably coupled with said peptide sequence via an amide bond at the N-terminal.

The modified peptides according to the invention, wherein the conformationally rigid moiety is the formula referenced 60 in the description, are of a particular interest.

The modified peptides of the present invention can be administered in various ways, such as for example, intravenously, subcutaneously, intradermally,

transdermally, intraperitoneally, orally, or topically. The modified peptides of the present invention can also be administered by inhalation, when in a powder form or aerosol form. Furthermore, pharmaceutically acceptable carriers for delivery of modified peptides of the present invention include, without limitation, liposome, nanosome, patch, implant or any delivery devices.

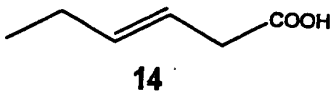
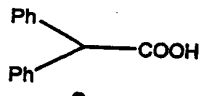
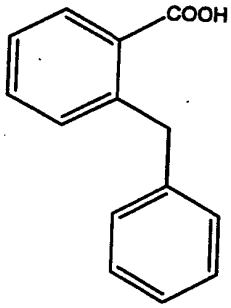
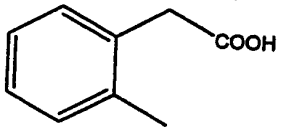
In addition to the carboxy and amino groups present at the C- and N-terminals respectively of the peptide, other carboxy and amino sites can be available on the peptide chain. For example, if the peptide chain comprises amino acids provided with a carboxylic acid side chain such as aspartic acid and glutamic acid, additional carboxy sites will therefore be available on the chain for amidation. Should the peptide chain comprise amino acids with a carboxamide side chain such as asparagine and glutamine, these also provide additional carboxy groups for amidation by a conformationally rigid moiety, provided that they are accessed synthetically via the corresponding aspartic and glutamic acids. Further, if the peptide comprises amino acids provided with a basic side chain such as arginine, histidine or lysine, additional amino sites will then be available on the chain for amidation by a conformationally rigid moiety. The peptide chain may also include both acidic and basic amino acids, meaning that the conformationally rigid substituents could be coupled to the peptide chain via the N-terminal, the C-terminal, a carboxy site on the peptide chain, an amino site on the peptide chain, or a plurality of these sites.

The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

EXAMPLE 1

Synthesis of GLP-1 analogs

In accordance with the present invention, at least one of the following conformationally rigid moiety is coupled with the GLP-1 peptide sequence via an amide bond at different positions as follows.

Position	conformationally rigid moieties
His_1	 14  2  60  63  13

hGLP-1 (7-37) analogs synthesis

hGLP-1 (7-37) derivatives modified at the amino terminus with rigid hydrophobic moieties were synthesized using Fmoc chemistry (1), on the Symphony apparatus (Rainin Instrument Co., Inc.). Fmoc-Gly-Wang resin (0.70mmole/g) and five

equivalents of reagents (100µm scale, amino acids concentration of 200mM), were used with a time coupling of 30 minutes. The reactions have been monitored by the Kaiser test. The three conformationally rigid moieties introduced at the N-terminus of the hGLP-1 (7-37) are:

- **Peptide # 1** = (O-Tolylacetic acid-His^7)-hGLP-1 (7-37) [O-Tolylacetic acid (13) (10 equivalents per coupling; coupling time 45 min)]
- **Peptide # 2** = ((+,-)-*cis*-2-Ethylcyclopropylacetic acid -His^7)-hGLP-1 (7-37) [(+,-)-*cis*-2-Ethylcyclopropylacetic acid (60) (7.5 equivalents per coupling: coupling time 60 min)].

The peptides were cleaved using a TFA cocktail (92% TFA, 2% ethanedithiol, 2% thioanisole, 2% triisopropylsilane, 2% water, 2% (w/v) phenol) for 2 hours. All the analogs have been purified by reverse-phase HPLC. They have been analyzed by analytical HPLC and by MS (MALDI-TOF).

The synthesis of GLP-1 analogs is well known to the person skilled in the art and is further illustrated by the general references Fmoc Solid Phase Peptide Synthesis. A Practical Approach (2000). Chan, W.C. and White, P.D., Oxford University Press, New York, USA, 346p which are incorporated by reference.

Biological assess of GLP-1 analogs

Materials & Methods

Oral Glucose Tolerance Test (OGTT)

Six-week old female CD1 mice (Charles River) were fasted for at least 16 hours. Mice were given 1.5 mg of glucose per gram of body weight orally in water through a gastric gavage tube at t = o min and blood was collected from a tail vein at t = 0, 10, 20, 30, 60, 90 and 120 min for measurement of blood glucose using a glucose meter (Lifescan). Peptides or vehicle were injected subcutaneously 5 min prior to the glucose administration. Data were expressed as the area under the curve, calculated from the change (delta) in blood glucose for each time, using the trapezoidal rule. Therefore, the data represent the integrated increase in blood glucose over a 120 min period following glucose administration. Data presented are the mean ± SEM of 4 to 11 animals per group.

Test articles

All peptides, including wild-type GLP-1 (7-37), were tested in the OGTT test at 3 different concentrations: 1, 5 and 10 ug per mouse. In a first set of experiments (**study A**), peptide 3 was tested in comparison with vehicle and hGLP-1 (7-37). In a second set of experiments (**study B**), peptides 1 and 2 were tested in comparison with vehicle and hGLP-1 (7-37).

wt GLP1: hGLP(7-37)

Peptide #1: (O-Tolylacetic acid-His^7)-hGLP-1 (7-37)

Peptide #2: ((+,-)-cis-2-Ethylcyclopropylacetic acid-His^7)-hGLP-1 (7-37)

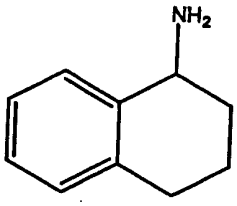
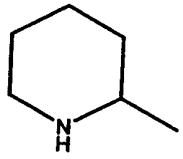
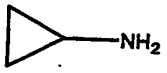
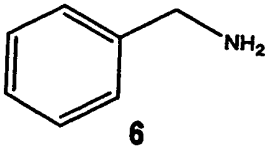
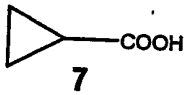
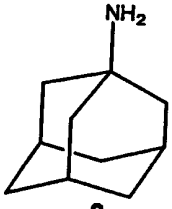
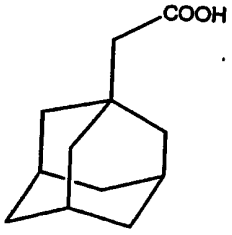
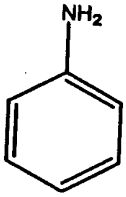
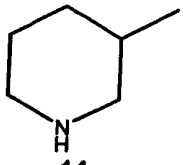
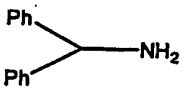
Peptide #3: (Hexenoyl-trans-3-His^7)-hGLP-1 (7-37)

Results and conclusions

Results are shown in Fig I(study A) and Fig.II (study B)

In studies A and B, administration of vehicle resulted in a similar integrated response in glucose levels (study A: 380 ± 57 vs study B: 309 ± 68 mMx120 min), illustrating the validity and reproducibility of the methodology. Although wt GLP-1 induced a dose-related decrease in the glucose response, this peptide was not able to completely suppress the glucose response at any dose, which might be

interpreted as a limitation in its potential clinical usefulness. In contrast, peptide 3 (study A, Fig.1) was able to completely abolish the glucose response, but only at the 10 ug dose (9 ± 26 mMx120 min). Surprisingly, peptide 2 (study B, Fig.2) was even more potent than peptide 3, being able to totally prevent the glucose response both at the 5 ug and the 10 ug doses (5 ug: -17 ± 67 mMx120 min; 10 ug: 61 ± 64 mMx120 min). In conclusion, the GLP-1 analog corresponding to peptide 2 was identified with marked increased biological potency over the wild type GLP-1 (7-37), because of this increased potency, this peptide may have clinical usefulness in treating states of insulin resistance associated with pathologies such as type II diabetes.

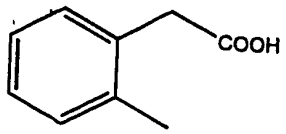
Position	conformationally rigid moieties
Glu_3	 3  4
Asp_9	 5  6
$His_1 + Glu_3$	 7  8
$His_1 + Asp_9$	 9  10
$Glu_3 + Asp_9$	 11  12

EXAMPLE 2

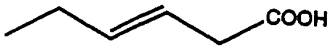
PTH 1-34 analogs

In accordance with the present invention, at least one of the following conformationally rigid moiety is coupled with the PTH 1-34 peptide sequence via an amide bond at different positions as follows.

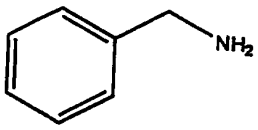
Position	conformationally rigid moieties

Ser_1 

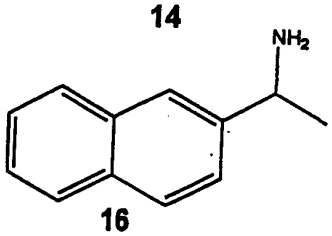
13



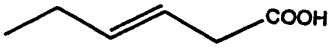
14

Glu_4 

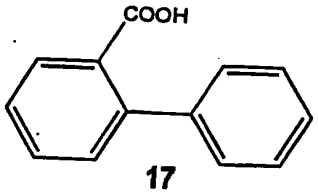
15



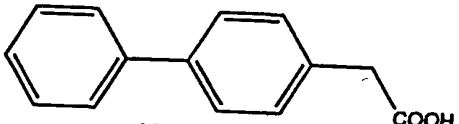
16

Lys_{26} 

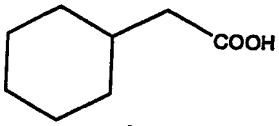
14



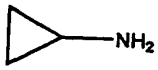
17

Lys_{27} 

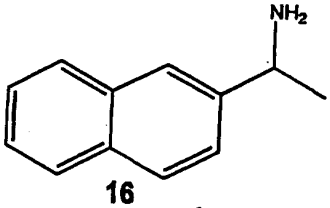
18



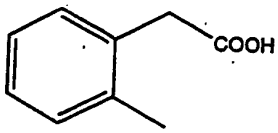
19

Asp_{30} 

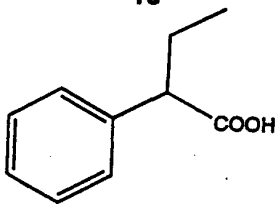
5



16

Ser_1+Lys_{27} 

13

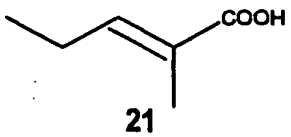
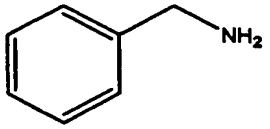
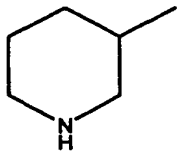
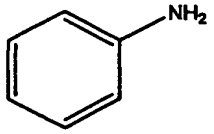
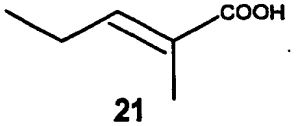
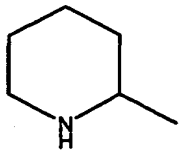


20

EXAMPLE 3

Somatostatin analogs

In accordance with the present invention, at least one of the following conformationally rigid moiety is coupled with the somatostatin peptide sequence via an amide bonds at different position as follows.

Position	conformationally rigid moieties
Ala_1	 21
Asp_5	 15  11
Cys_{14}	 10
Ala_1+Cys_{14}	 21  4

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications, and this application is intended to cover any variations, uses or adaptations of the invention following, in general, the principles of the invention, and including such

departures from the present description as come within known or customary practice within the art to which the invention pertains, and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A peptide of formula X_n-R_1 wherein:

- R_1 is a peptide sequence, a functional analog thereof or a fragment thereof;

each X can be identical or independent from the others and is selected from the following list constituted by conformationally rigid moieties:

i) a straight, substituted C_1-C_{10} alkyl;

ii) a branched, substituted C_1-C_{10} alkyl;

iii) a straight or branched, unsubstituted or substituted C_1-C_{10} alkene;

iv) a straight or branched, unsubstituted or substituted C_1-C_{10} alkyne;

v) an unsubstituted or substituted, saturated or unsaturated C_3-C_{10} cycloalkyl or heterocycloalkyl wherein the heteroatom is O, S or N;

vi) an unsubstituted or substituted C_5-C_{14} aryl or heteroaryl wherein the heteroatom is O, S or N;

wherein the substituent in the definitions i) to vi) comprises one or more

a) straight or branched C_1-C_6 alkyl;

b) straight or branched C_1-C_6 alkene;

c) straight or branched C_1-C_6 alkyne;

d) C_3-C_{10} cycloalkyl or heterocycloalkyl wherein at least 2 carbon atoms are optionally connected to the C_1-C_{10} alkyl, C_1-C_{10} alkene, C_1-C_{10} alkyne, C_3-C_{10} cycloalkyl or heterocycloalkyl, and C_5-C_{14} aryl or heteroaryl; or

e) C_5-C_{14} aryl or heteroaryl wherein at least 2 carbon atoms of the aryl or heteroaryl are optionally connected to the C_1-C_{10} alkyl, C_1-C_{10} alkene, C_1-C_{10}

alkyne, C_3-C_{10} cycloalkyl or heterocycloalkyl, and C_5-C_{14} aryl or heteroaryl, said group X also comprising at least one group selected from:

α) a carboxy or an amino group for coupling with the peptide sequence via an amide bond at the N-terminal of the peptide sequence, the C-terminal of the peptide sequence, at an available carboxy or amino site on the peptide sequence chain, and combinations thereof; and

β) a carboxy group for coupling with the peptide sequence via an ester bond at an available hydroxy site on the peptide sequence chain, and combinations thereof;

wherein,

n is any digit between 1 to 5;

and any isomers thereof, including cis and trans configurations, epimers, enantiomers, diastereoisomers, and racemic mixtures,

the peptides defined in claim 1 of U.S. Patent No. 6,020,311 being excluded.

2. A peptide as claimed in claim 1 wherein the peptide sequence is selected from the group consisting of Growth hormone releasing factor (GRF), Somatostatin, Glucagon-like peptide 1 (7-37), amide human (GLP-1) hGLP-1 (7-36) NH_2, Parathyroid hormone fragments (PTH 1-34), Adrenocorticotropic hormone (ACTH), Osteocalcin, Calcitonin, Corticotropin releasing factor, Dynorphin A, β–Endorphin, Big Gastrin-1, GLP-2, Luteinizing hormone-releasing hormone, Melanocyte Stimulating Hormone (MSH), Atrial Natriuretic Peptide, Neuromedin B, Human

Neuropeptide Y, Human Orexin A, Human Peptide YY, Human Secretin, Vasoactive Intestinal peptide (VIP), Antibiotic peptides (Magainin 1, Magainin 2, Cecropin A, and Cecropin B), Substance P (SP), Beta Casomorphin-5, Endomorphin-2, Procolipase, Enterostatin, gastric inhibitory peptide, Chromogranin A, Vasostatin I & II, Procalcitonin, ProNCT, CGRP (Calcitonin Gene Related Peptide), IL8 (monocyte-derived), GCP-2, PF4, IP-10, MIG, SDF-1α, GRO-α, I-TAC, RANTES, LD78, MIP-1α, MCP-1, MCP-2, MCP-3, MCP-4, Eotaxin, MDC, and functional analogs and derivatives or fragments thereof.

3. A peptide as claimed in claim 1 or 2 wherein the conformationally rigid moiety comprises at least a double bond, a triple bond or a saturated or unsaturated ring.

4. A peptide as claimed in any one of claims 1 to 3 wherein the conformationally rigid moiety comprises one or more of the structures of Formula 1 to 63 as defined in the description.

5. A peptide as claimed in any one of claims 1 to 4 wherein the peptide sequence is selected from the group consisting of:

Growth hormone releasing factor (GRF):

Xaa_1-Xaa_2-Asp-Ala-Ile-Phe-Thr-Xaa_8-Ser-Tyr-Arg-Lys-Xaa_{13}-Leu-Xaa_{15}-Gln-Leu-Xaa_{18}-Ala-Arg-Lys-Leu-Leu-Xaa_{24}-Xaa_{25}-Ile-Xaa_{27}-Xaa_{28}-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu-NH_2

wherein,

Xaa_1 is Tyr or His;

Xaa_2 is Val or Ala;
Xaa_8 is Asn or Ser;
Xaa_{13} is Val or Ile;
Xaa_{15} is Ala or Gly;
Xaa_{18} is Ser or Tyr;
Xaa_{24} is Gln or His;
Xaa_{25} is Asp or Glu;
Xaa_{27} is Met, Ile or Nle; and
Xaa_{28} is Ser or Asn;

Somatostatin:

Ala_1-Gly-Cys-Lys-Asn-Phe-Phe-Trp
|
Cys_{14}-Ser-Xaa_{12}-Phe-Thr-Lys
|

wherein,

Xaa_{12} is Tyr or Ser;

Glucagon-like peptide 1 (7-37), (amide human (hGLP-1)):

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly-OH(NH_2)

Parathyroid hormone fragments (PTH 1-34):

Xaa_1-Val-Ser-Glu-Xaa_5-Gln-Xaa_7-Met-His-Asn-Leu-Gly-Xaa_{13}-His-Xaa_{15}-Xaa_{16}-Xaa_{17}-Xaa_{18}-Glu-Arg-Xaa_{21}-Xaa_{22}-Trp-Leu-Xaa_{25}-Xaa_{26}-Lys-Leu-Gln-Asp-Val-His-Xaa_{33}-Xaa_{34}-NH_2

wherein,

Xaa_1 is Ser or Ala;

Xaa_5 is Ile or Met;

Xaa_7 is Leu or Phe;

Xaa_{13} is Lys or Glu;

Xaa_{15} is Leu or Arg;

Xaa_{16} is Asn or Ala or Ser or His;

Xaa_{17} is Ser of Thr;

Xaa_{18} is Met or Val or Leu;

Xaa_{21} is Val or met or Gln;

Xaa_{22} is Glu or Gln or Asp;

Xaa_{25} is Arg or Gln;

Xaa_{26} is Lys or Met;

Xaa_{33} is Asn or Ser; and

Xaa_{34} is Phe or Ala;

Adrenocorticotropic hormone (ACTH):

Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Xaa_{13}-Gly-Xaa_{15}-Lys-Arg-Arg-Pro-Xaa_{20}-Lys-Val-Tyr-Pro-Asn-Xaa_{26}-Xaa_{27}-Xaa_{28}-Xaa_{29}-Glu-Xaa_{31}-Xaa_{32}-Glu-Xaa_{34}-Xaa_{35}-Xaa_{36}-Xaa_{37}-Glu-Xaa_{39}-NH_2

wherein,

Xaa_{13} is Val or Met;

Xaa_{15} is Lys or Arg;

Xaa_{20} is Val or Ile;

Xaa_{26} is Gly or Ser;

Xaa_{27} is Ala or Phe or Val;

Xaa_{28} is Glu or Gln;

Xaa_{29} is Asp or Asn or Glu;

Xaa_{31} is Ser or Thr;

Xaa_{32} is Ala or Val or Ser;

Xaa_{34} is Ala or Asn or Gly;

Xaa_{35} is Phe or Met;

Xaa_{36} is Pro or Gly;

Xaa_{37} is Leu or Val or Pro; and

Xaa_{39} is Phe or Val or Leu;

Osteocalcin:

Tyr-Leu-Xaa_{52}-Xaa_{53}-Xaa_{54}-Leu-Gly-Ala-Pro-Xaa_{59}-Pro-Tyr-Pro-Asp-Pro-Leu-Glu-Pro-Xaa_{68}-Arg-Glu-Val-Cys-Glu-Leu-Asn-Pro-Xaa_{77}-Cys-Asp-Glu-Leu-Ala-Asp-His-Ile-Gly-Phe-Gln-Xaa_{89}-Ala-Tyr-Xaa_{92}-Arg-Xaa_{94}-Tyr-Gly-Xaa_{97}-Val-NH_2

wherein,

Xaa_{52} is Tyr or Asp or Asn;

Xaa_{53} is Gln or His or Asn;

Xaa_{54} is Trp or Gly;

Xaa_{59} is Val or Ala;

Xaa_{68} is Arg or Lys or His;

Xaa_{77} is Asp or Asn;

Xaa_{89} is Glu or Asp;

Xaa_{92} is Arg or Lys;

Xaa_{94} is Phe or Ile; and

Xaa_{97} is Pro or Thr;

Calcitonin:

Cys-Xaa_{86}-Xaa_{87}-Leu-Ser-Thr-Cys-Xaa_{92}-Leu-Gly-Xaa_{95}-Xaa_{96}-Xaa_{97}-Xaa_{98}-Xaa_{99}-Xaa_{100}-Xaa_{101}-Xaa_{102}-Xaa_{103}-Xaa_{104}-Thr-Xaa_{106}-Xaa_{107}-Xaa_{108}-Xaa_{109}-Xaa_{110}-Xaa_{111}-Gly-Xaa_{113}-Xaa_{114}-Xaa_{115}-Pro-NH_2

wherein,

Xaa_{86} is Gly or Ser or Ala;

Xaa_{87} is Asn or Ser;

Xaa_{92} is Met or Val;

Xaa_{95} is Thr or Lys;

Xaa_{96} is Tyr or Leu;

Xaa_{97} is Thr or Ser;

Xaa_{98} is Gln or Lys;

Xaa_{99} is Asp or Glu;

Xaa_{100} is Phe or Leu;

Xaa_{101} is Asn or His;

Xaa_{102} is Lys or Asn;

Xaa_{103} is Phe or Leu;

Xaa_{104} is His or Gln;

Xaa_{106} is Phe or Tyr;

Xaa_{107} is Pro or Ser;

Xaa_{108} is Gln or Gly or Arg;

Xaa_{109} is Thr or Ile;

Xaa_{110} is Ala or Gly or Ser or Asp or Asn;

Xaa_{111} is Ile or Phe or Val or Thr;

Xaa_{113} is Val or Ala or Ser;

Xaa_{114} is Gly or Glu; and

Xaa_{115} is Ala or Thr;

Corticotropin releasing factor:

Ser-Glu-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Xaa_{101}-Xaa_{102}-Ala-Glu-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Met-Glu-Ile-Ile-NH_2

wherein,

Xaa_{101} is Ala or Pro; and

Xaa_{102} is Arg or Gly;

Dynorphin A:

H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH

β-Endorphin:

H-Tyr-Gly-Gly-Phe-Met-Thr-Xaa_{243}-Glu-Xaa_{245}-Ser-Gln-Thr-Pro-Leu-Xaa_{251}-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Xaa_{259}-Lys-Asn-Xaa_{262}-Xaa_{263}-Lys-Lys-Gly-Xaa_{267}-OH

wherein,

Xaa_{243} is Ser or Pro;

Xaa_{245} is Lys or Arg;

Xaa_{251} is Val or Met;

Xaa_{259} is Ile or Val;

Xaa_{262} is Ala or Thr or Ser or Val;

Xaa_{263} is Tyr or His; and

Xaa_{267} is Glu or Leu or Gln or His;

Big Gastrin-1:

pXaa_{59}-Leu-Gly-Xaa_{62}-Gln-Xaa_{64}-Xaa_{65}-Xaa_{66}-Xaa_{67}-Xaa_{68}-Xaa_{69}-Ala-Asp-Xaa_{72}-Xaa_{73}-Lys-Lys-Xaa_{76}-Xaa_{77}-Pro-Xaa_{79}-Xaa_{80}-Glu-Xaa_{82}-Glu-Glu-Xaa_{85}-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2

wherein,

Xaa_{59} is Glu or Gln;

Xaa_{62} is Pro or Leu;

Xaa_{64} is Gly or Asp;

Xaa_{65} is Pro or Ser;

Xaa_{66} is Pro or Gln;

Xaa_{67} is His or Gln;

Xaa_{68} is Leu or Met or Phe or Gln;

Xaa_{69} is Val or Ile;

Xaa_{72} is Pro or Leu;

Xaa_{73} is Ser or Ala;

Xaa_{76} is Gln or Glu;

Xaa_{77} is Gly or Arg;

Xaa_{79} is Trp or Pro or Arg;

Xaa_{80} is Leu or Val or Met;

Xaa_{82} is Glu or Lys; and

Xaa_{85} is Glu or Ala;

GLP-2:

His-Ala-Asp-Gly-Ser-Phe-Xaa_{152}-Xaa_{153}-Xaa_{154}-Xaa_{155}-Xaa_{156}-Xaa_{157}-Xaa_{158}-Leu-Asp-Xaa_{161}-Xaa_{162}-Ala-Xaa_{164}-Xaa_{165}-Xaa_{166}-Phe-Xaa_{168}-Xaa_{169}-Trp-Xaa_{171}-Xaa_{172}-Xaa_{173}-Thr-Xaa_{175}-Xaa_{176}-Xaa_{177}-Xaa_{178};

wherein,

Xaa_{152} is Ser or Thr;

Xaa_{153} is Asp or Ser;

Xaa_{154} is Glu or Asp;

Xaa_{155} is Met or Phe;

Xaa_{156} is Asn or Ser;

Xaa_{157} is Thr or Lys;

Xaa_{158} is Ile or Val or Ala;

Xaa_{161} is Asn or Ile or His or Ser;

Xaa_{162} is Leu or Lys;

Xaa_{164} is Ala or Thr;

Xaa_{165} is Arg or Gln or Lys;

Xaa_{166} is Asp or Glu;

Xaa_{168} is Ile or Leu;

Xaa_{169} is Asn or Asp;

Xaa_{171} is Leu or Ile;

Xaa_{172} is Ile or Leu;

Xaa_{173} is Gln or Asn or His;

Xaa_{175} is Lys or Pro;

Xaa_{176} is Ile or Val;

Xaa_{177} is Thr or Lys; and

Xaa_{178} is Asp or Glu;

Luteinizing hormone-releasing hormone:

Xaa_1-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-OH

wherein,

Xaa_1 is pGlu, 5-oxoPro or Gln.

Melanocyte Stimulating Hormone (MSH):

Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2

Atrial Natriuretic Peptide:

H-Ser-Leu-Arg-Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Xaa_{135}-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Xaa_{142}-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr-OH

wherein,

Xaa_{135} is Met or Ile; and

Xaa_{142} is Gly or Ser;

Neuromedin B:

H-Gly-Asn-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH_2

Human Neuropeptide Y:

H-Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-asp-Met-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2

Human Orexin A:

pGlu-Pro-Leu-Pro-Asp-Cys-Cys-Arg-Gln-Lys-Thr-Cys-Ser-Cys-Arg-Leu-Tyr-Glu-Leu-Leu-His-Gly-Ala-Gly-Asn-His-Ala-Ala-Gly-Ile-Leu-Thr-Leu-NH_2

Human Peptide YY:

H-Tyr-Pro-Ile-Lys-Pro-Glu-Ala-Pro-Gly-Glu-Asp-Ala-Ser-Pro-Glu-Glu-Leu-Asn-Arg-Tyr-Tyr-Ala-Ser-Leu-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH_2

Human Secretin:

H-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Glu-Gly-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH_2

Vasoactive Intestinal peptide (VIP):

H-His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2

Antibiotic peptides such as:

Magainin 1:

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Gly-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-Met-Lys-Ser

Magainin 2:

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-Met-Asn-Ser

Cecropin A:

Lys-Trp-Lys-Val-Phe-Lys-Lys-Ile-Glu-Lys-Val-Gly-Gln-Ala-Thr-Gln-Ile-Ala-Lys

Cecropin B:

Lys-Trp-Lys-Val-Phe-Lys-Lys-Ile-Glu-Lys-Met-Gly-Arg-Asn-Ile-Arg-Asn-Gly-Ile-Val-Lys-Ala-Gly-Pro-Ala-Ile-Ala-Val-Leu-Gly-Glu-Ala-Lys-Ala-Leu .

Substance P (SP):

Arg-Pro-Leu-Pro-Gln-Glu-Phe-Phe-Gly-Leu-Met-amide

Beta Casomorphin-5:

Tyr-Pro-Phe-Pro-Gly

Endomorphin-2:

Tyr-Pro-Phe-Phe-NH2

Procolipase:

100 aa peptide (X1-Pro-X2-Pro-Arg....)

Enterostatin:

Val-Pro-Asp-Pro-Arg

Gastrin Inhibitory Peptide:

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala- Met-Asp-Lys-Ile-His-Gln-Gln-Asp-Phe- Val-Asn-Trp-Leu- Leu-Ala-Gln-Lys-Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn- Ile-Thr-Gln

Chromogranin A

Vasostatin I

Vasostatin II:

Leu Pro Val Asn Ser Pro Met Asn Lys Gly Asp Thr Glu Val Met Lys Cys Ile Val Glu Val Ile Ser Asp Thr Leu Ser Lys Pro Ser Pro Met Pro Val Ser Gln Glu Cys Phe Glu Thr Leu Arg Gly Asp Glu Arg Ile Leu Ser Ile Leu Arg His Gln Asn Leu Leu

Lys Glu Leu Gln Asp Leu Ala Leu Gln Gly Ala Lys Glu Arg Ala His Gln Gln Lys Lys His Ser Gly Phe Glu Asp Glu Leu Ser Glu Val Leu Glu Asn Gln Ser Ser Gln Ala Glu Leu Lys Glu Ala Val Glu Glu Pro Ser Ser Lys Asp Val Met Glu

Procalcitonin

ProNCT

ProCGRP

Chemokine family:

CXC-group:

IL8(monocyte-derived):

SerAlaLysGluLeuArgCysGlnCys...

GCP-2:

GlyProValSerAlaValLeuThrGluLeuArgCysThrCys...

PF4:

GluAlaGluGluAspGlyAspLeuGlnCysLeuCys...

IP-10:

ValProLeuSerArgThrValArgCCysThrCys...

MIG:

ThrProValValArgLysGlyArgCysSerCys...

SDF-1α:

LysProValSerLeuSerTyrArgCysProCys...

GRO-α:

AlaProLeuAlaThrGluLeuArgCysGlnCys...

I-TAC:

PheProMetPheLysLysGlyArgCysLeuCys...

CC-group:

RANTES:

SerProTyrSerSerAspThrThrProCys...

LD78:

AlaProLeuAlaAlaAspThrProThrAlaCys...

MIP-1α:

AlaProMetGlySerAspProProThrAlaCys...

MCP-1:

GlnProAspAlaIleAsnAlaProValThrCys...

MCP-2:

GlnProSerAspValSerIleProIleThrCys...

MCP-3:

GlnProValGlyIleTAsnSeerThrThrCys...

MCP-4:

GlnProAspAlaLeuAspValProSerThrCys...

Eotaxin:

GlyProAlaSerValProThrThrCys...

MDC:

GlyProTyrGlyAlaAsnMetGluAspSerValCys...

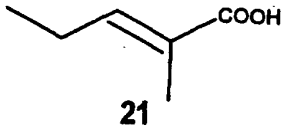
and functional analogs and derivatives or fragments thereof.

6. A peptide according to claim 5 wherein the peptide sequence is the sequence of a natural peptide and functional analog or a fragment thereof or a clinically safe and acceptable derivative or analog thereof.

7. A peptide as claimed in claim 1 wherein the peptide sequence is Somatostatin and at least one conformationally rigid moiety is coupled with said somatostatin peptide sequence via an amide bond at different positions as follows:

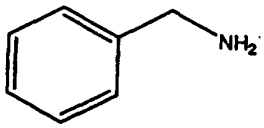
Position, conformationally rigid moieties

Ala_1

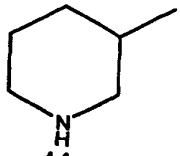


21

Asp_5

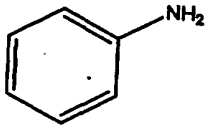


15



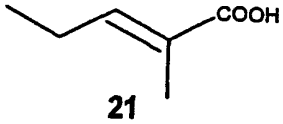
11

Cys_{14}

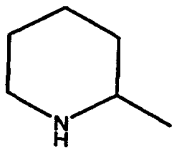


10

Ala_1+Cys_{14}



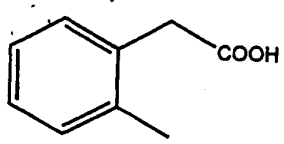
21



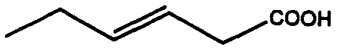
4

8. A peptide as claimed in claim 1 wherein the peptide sequence is PTH 1-34 and at least one conformationally rigid moiety is coupled with said PTH 1-34 peptide sequence via an amide bond at different positions as follows:

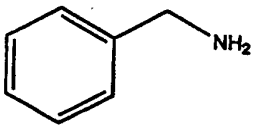
Position	conformationally rigid moieties

Ser_1 

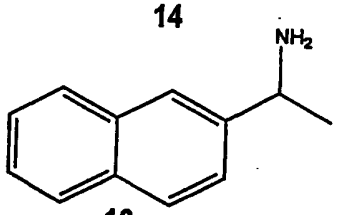
13



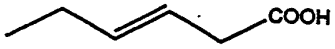
14

Glu_4 

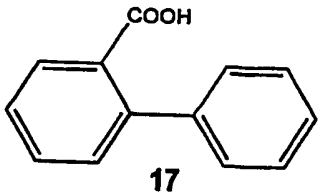
15



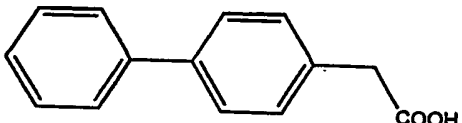
16

Lys_{26} 

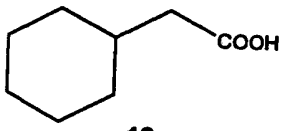
14



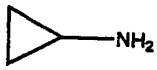
17

Lys_{27} 

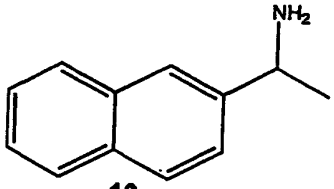
18



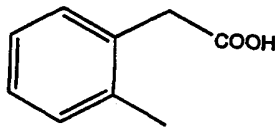
19

Asp_{30} 

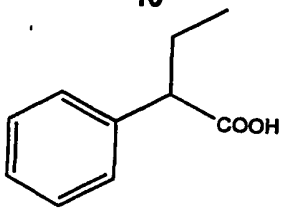
5



16

Ser_1+Lys_{27} 

13

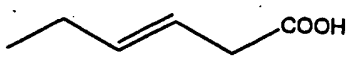


20

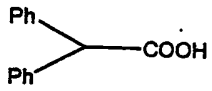
9. A peptide as claimed in claim 1 wherein said peptide sequence is GLP-1 and at least one conformationally rigid moiety is coupled with said GLP-1 peptide sequence via an amide bond at different positions as follows:

Position	**conformationally rigid moieties**

His_1

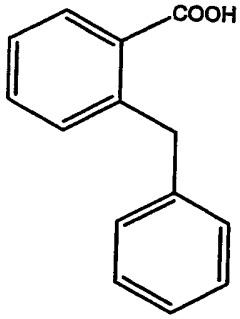


14

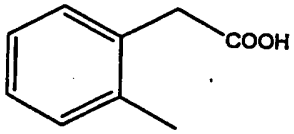


2

60

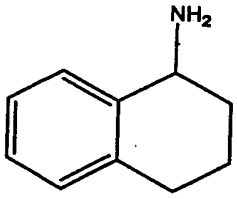


63

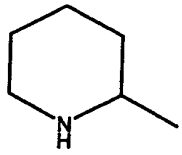


13

Glu_3

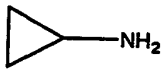


3

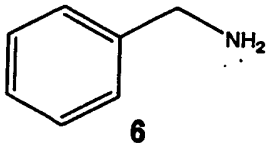


4

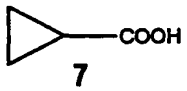
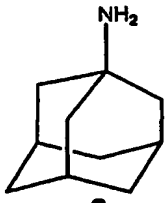
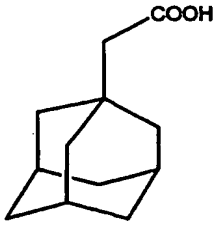
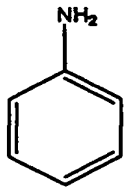
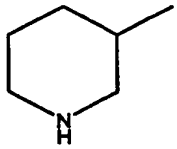
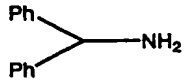
Asp_9



5



6

Position	conformationally rigid moieties	
$His_1 + Glu_3$	 7	 8
$His_1 + Asp_9$	 9	 10
$Glu_3 + Asp_9$	 11	 12

10. A peptide as claimed in claim 1 wherein said peptide sequence is GLP-2 and at least one conformationally rigid moiety is coupled with said GLP-2 peptide sequence via an amide or ester bond at different positions of the peptide sequence.

11. A peptide as claimed in claim 1 wherein said peptide sequence is Enterostatin and at least one conformationally rigid moiety is coupled with said Enterostatin peptide sequence via an amide bond at different positions of the peptide sequence.

12. A peptide as claimed in claim 1 wherein said peptide sequence is NPY and at least one conformationally rigid moiety is coupled with said NPY peptide sequence via an amide or ester bond at different positions of the peptide sequence.

13. A peptide as claimed in claim 1 wherein said peptide sequence is NPYY and at least one conformationally rigid moiety is coupled with said NPYY peptide sequence via an amide or ester bond at different positions of the peptide sequence.

14. A peptide as claimed in claim 1 wherein said peptide sequence is Secretin and at least one conformationally rigid moiety is coupled with said Secretin peptide sequence via an amide or ester bond at different positions of the peptide sequence.

15. A peptide as claimed in claim 1 wherein said peptide sequence is Vasoactive Intestinal Peptide and at least one conformationally rigid moiety is coupled with said

Vasoactive Intestinal Peptide sequence via an amide or ester bond at different positions of the peptide sequence.

16. A peptide as claimed in claim 1 wherein said peptide sequence is Gastrin Inhibitory Peptide and at least one conformationally rigid moiety is coupled with said Gastrin Inhibitory Peptide sequence via an amide or ester bond at different positions of the peptide sequence.

17. A peptide as claimed in claim 1 wherein said peptide sequence is Vasostatin II and at least one conformationally rigid moiety is coupled with said Vasostatin II peptide sequence via an amide or ester bond at different positions of the peptide sequence.

18. A peptide as claimed in claim 1 wherein said peptide sequence is RANTES and at least one conformationally rigid moiety is coupled with said RANTES peptide sequence via an amide or ester bond at different positions of the peptide sequence.

19. A peptide as claimed in claim 1 wherein said peptide sequence is Eotaxin and at least one conformationally rigid moiety is coupled with said Eotaxin peptide sequence via an amide or ester bond at different positions of the peptide sequence.

20. A peptide as in any one of claims 1 to 18, wherein said conformationally rigid moiety is coupled with said peptide sequence via an amide or ester bond at the N-terminal.

21. A peptide according to any one of claims 8 to 19, wherein the conformationally rigid moiety has the formula 60 referenced in the description.

22. A peptide according to claim 20, wherein the peptide sequence is GLP-1.

23. Use of the peptide according to claim 22 in the treatment of glucose intolerance associated or not with insuline resistance pathologies.

24. Use according to claim 23 in the treatment of type II diabetes.

25. A peptide according to claim 1 wherein said peptide sequence is CGRP and at least one conformationally rigid moiety is coupled with said CGRP peptide sequence via an amide or ester bond at different positions of the peptide sequence.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number
WO 02/010195 A3

(51) International Patent Classification[7]: C07K 14/605, 14/00, 14/47, A61K 38/04, C07K 14/635, 14/655

(21) International Application Number: PCT/CA01/01119

(22) International Filing Date: 2 August 2001 (02.08.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/222,619 2 August 2000 (02.08.2000) US

(71) Applicant *(for all designated States except US)*: **THERATECHNOLOGIES INC.** [CA/CA]; 2310, Alfred-Nobel Boulevard, Saint-Laurent, Québec H4S 2A4 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **GRAVEL, Denis** [CA/CA]; 207, des Pyrénées, St-Lambert, Québec J4S 1L3 (CA). **HABI, Abdelkrim** [CA/CA]; 7961, Champ d'Eau, Anjou, Québec H1J 1X4 (CA). **ABRIBAT, Thierry** [CA/CA]; 4659, Hutchison, Montréal, Québec H2V 4A2 (CA).

(74) Agent: **SWABEY OGILVY RENAULT**; Suite 1600, 1981 McGill College Avenue, Montréal, Québec H3A 2Y3 (CA).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

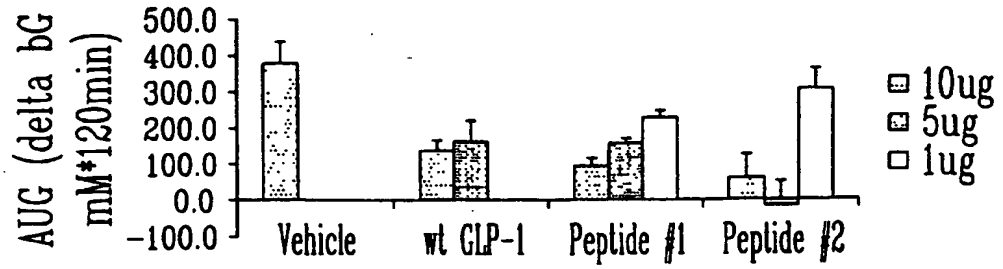
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
3 October 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MODIFIED PEPTIDES WITH INCREASED POTENCY



(57) Abstract: The present invention is concerned with modified biological peptides providing increased potency, prolonged activity and/or increased half-life thereof. The modification is made via coupling through an amide bond with at least one conformationally rigid substituent, either at the N-terminal of the peptide, the C-terminal of the peptide, on a free amino or carboxyl group along the peptide chain, or at a plurality of these sites. Those peptides exhibit clinical usefulness for example in treating states of insulin resistance associated with pathologies such as type II diabetes.

WO 02/010195 A3

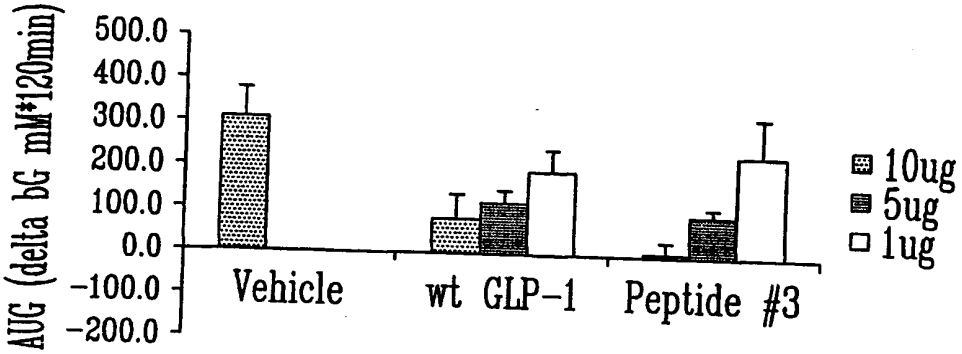

AUC (delta bG mM*120min)
500.0
400.0
300.0
200.0
100.0
0.0
-100.0
-200.0
Vehicle
wt GLP-1
Peptide #3
10ug
5ug
1ug

Fig. 1

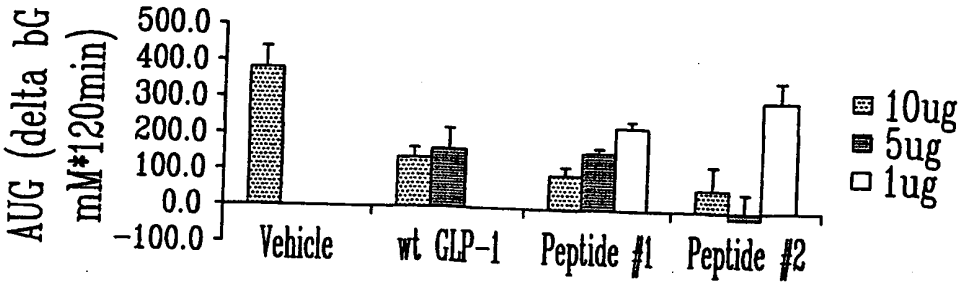

AUC (delta bG mM*120min)
500.0
400.0
300.0
200.0
100.0
0.0
-100.0
Vehicle
wt GLP-1
Peptide #1
Peptide #2
10ug
5ug
1ug

Fig. 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 01/01119

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/605 C07K14/00 C07K14/47 A61K38/04 C07K14/635
C07K14/655

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 14236 A (THERATECHNOLOGIES) 16 March 2000 (2000-03-16) the whole document	7-9,20, 21,23,24
A	WO 96 37514 A (THERATECHNOLOGIES) 28 November 1996 (1996-11-28) the whole document & US 6 020 311 A (THERATECHNOLOGIES) 1 February 2000 (2000-02-01) cited in the application	7-9,20, 21,23,24
A	WO 98 08871 A (NOVO NORDISK) 5 March 1998 (1998-03-05) the whole document	9,20,21, 23,24

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 July 2002	26/07/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Masturzo, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 01/01119

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 34332 A (SOCIETE DE CONSEILS, DE RECHERCHES ET D'APPLICATIONS SCIENTIFIQUES) 15 June 2000 (2000-06-15) the whole document	9,20,21, 23,24
A	WO 00 34331 A (SOCIETE DE CONSEILS ET D'APPLICATIONS SCIENTIFIQUES) 15 June 2000 (2000-06-15) the whole document	9,20,21, 23,24
A	WO 99 43707 A (NOVO NORDISK) 2 September 1999 (1999-09-02) the whole document	9,20,21, 23,24
A	US 5 093 233 A (M ROSENBLATT ET AL.) 3 March 1992 (1992-03-03) the whole document	8,20,21, 23,24
A	WO 98 01474 A (DOX-AL ITALIA) 15 January 1998 (1998-01-15) the whole document	7,20,21, 23,24
A	EP 0 187 622 A (SANDOZ-PATENT GMBH ET AL.) 16 July 1986 (1986-07-16) the whole document	7,20,21, 23,24
A	EP 0 030 920 A (CIBA-GEIGY) 24 June 1981 (1981-06-24) the whole document	7,20,21, 23,24

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-6, 10-25

Present claims 1-25 relate to an extremely large number of possible compounds and pertinent methods of use. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds and methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds of claim 7-9 and pertinent methods.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 01/01119

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 23-24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-6, 10-25
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ERNATIONAL SEARCH REPORT

Information on patent family members

ternational Application No
PCT/CA 01/01119

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0014236 A	16-03-2000	US 6020311 A	01-02-2000
		AU 5500799 A	27-03-2000
		BR 9913515 A	05-06-2001
		WO 0014236 A2	16-03-2000
		DE 1109909 T1	23-05-2002
		EP 1109909 A2	27-06-2001
		ES 2164626 T1	01-03-2002
WO 9637514 A	28-11-1996	AT 204881 T	15-09-2001
		AU 697119 B2	24-09-1998
		AU 5683396 A	11-12-1996
		BR 9608799 A	07-12-1999
		CA 2222068 A1	28-11-1996
		WO 9637514 A1	28-11-1996
		DE 69614849 D1	04-10-2001
		DE 69614849 T2	16-05-2002
		DK 828758 T3	07-01-2002
		EP 0828758 A1	18-03-1998
		ES 2163020 T3	16-01-2002
		JP 11505807 T	25-05-1999
		PT 828758 T	28-02-2002
		US 6020311 A	01-02-2000
		US 5939386 A	17-08-1999
		US 5861379 A	19-01-1999
WO 9808871 A	05-03-1998	AU 732957 B2	03-05-2001
		AU 3847897 A	19-03-1998
		AU 4112497 A	19-03-1998
		BR 9711437 A	18-01-2000
		CN 1232470 A	20-10-1999
		CZ 9900629 A3	14-07-1999
		WO 9808871 A1	05-03-1998
		WO 9808872 A1	05-03-1998
		EP 0944648 A1	29-09-1999
		EP 0929576 A1	21-07-1999
		HU 9903714 A2	28-03-2000
		JP 2000500505 T	18-01-2000
		JP 3149958 B2	26-03-2001
		JP 2000517308 T	26-12-2000
		JP 2001011095 A	16-01-2001
		NO 990950 A	28-04-1999
		PL 331896 A1	16-08-1999
		US 6268343 B1	31-07-2001
		US 2001011071 A1	02-08-2001
		US 2002025933 A1	28-02-2002
WO 0034332 A	15-06-2000	AU 1751200 A	26-06-2000
		BR 9916027 A	28-08-2001
		CN 1342166 T	27-03-2002
		CZ 20011895 A3	12-12-2001
		EP 1137666 A1	04-10-2001
		NO 20012787 A	06-08-2001
		WO 0034332 A1	15-06-2000
WO 0034331 A	15-06-2000	AU 1973600 A	26-06-2000
		BR 9915961 A	21-08-2001
		CN 1329620 T	02-01-2002
		CZ 20011748 A3	13-02-2002

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 01/01119

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0034331 A		WO 0034331 A2	15-06-2000
		EP 1137667 A2	04-10-2001
		NO 20012786 A	20-07-2001
WO 9943707 A	02-09-1999	AU 2610699 A	15-09-1999
		AU 2610799 A	15-09-1999
		AU 2610899 A	15-09-1999
		WO 9943706 A1	02-09-1999
		WO 9943341 A1	02-09-1999
		WO 9943707 A1	02-09-1999
		EP 1060191 A1	20-12-2000
		EP 1061946 A1	27-12-2000
		EP 1062240 A1	27-12-2000
		JP 2002504518 T	12-02-2002
		JP 2002512175 T	23-04-2002
		JP 2002506792 T	05-03-2002
		US 6268343 B1	31-07-2001
		US 2001011071 A1	02-08-2001
		ZA 9901569 A	27-08-1999
		ZA 9901570 A	02-09-1999
US 5093233 A	03-03-1992	NONE	
WO 9801474 A	15-01-1998	IT MI961408 A1	08-01-1998
		AU 3693097 A	02-02-1998
		WO 9801474 A2	15-01-1998
		EP 0914342 A2	12-05-1999
EP 187622 A	16-07-1986	DE 3511206 A1	09-10-1986
		AU 5184886 A	10-07-1986
		DK 4686 A	08-07-1986
		EP 0187622 A2	16-07-1986
		ES 550692 D0	01-05-1987
		ES 8705469 A1	16-07-1987
		FI 860029 A	08-07-1986
		GR 860010 A1	30-04-1986
		HU 42101 A2	29-06-1987
		JP 61161300 A	21-07-1986
		US 4728638 A	01-03-1988
		ZA 8600115 A	26-08-1987
EP 30920 A	24-06-1981	AT 6251 T	15-03-1984
		AU 544256 B2	23-05-1985
		AU 6534580 A	18-06-1981
		CA 1161033 A1	24-01-1984
		DD 154098 A5	24-02-1982
		DE 3066654 D1	22-03-1984
		DK 532180 A	15-06-1981
		EP 0030920 A2	24-06-1981
		ES 497696 D0	16-11-1981
		ES 8200861 A1	16-02-1982
		FI 803849 A	15-06-1981
		GR 73621 A1	26-03-1984
		JP 56092850 A	27-07-1981
		KR 8500415 B1	26-03-1985
		KR 8500414 B2	26-03-1985
		NO 803757 A	15-06-1981
		NZ 195817 A	06-07-1984

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 01/01119

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 30920 A		PT 72191 A ,B	01-01-1981
		US 4369179 A	18-01-1983
		ZA 8007796 A	27-01-1982